JC03 Rec'd PCT/PTO 1 SFP 200

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK ATTORNEY'S DOCKET NUMBER OFFICE (REV 11-2000) 280502000200 TRANSMITTAL LETTER TO THE UNITED STATES U S. APPLICATION NO (If known, see 37 CFR 1 5) DESIGNATED/ELECTED OFFICE (DO/EO/US) 09/936887 CONCERNING A FILING UNDER 35 U.S.C. § 371 INTERNATIONAL APPLICATION NO INTERNATIONAL FILING DATE PCT/CA00/00266 March 12, 1999 March 13, 2000 TITLE OF INVENTION METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA APPLICANT(S) FOR DO/EO/US Chaim M. ROIFMAN, et al. Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information This is a FIRST submission of items concerning a filing under 35 U S.C. 371. × This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371 2. This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) 3 indicated below The US has been elected by the expiration of 19 months from the priority date (PCT Article 31) 4 A copy of the International Application as filed (35 U.S.C. 371(c)(2)) \boxtimes is attached hereto (required only if not communicated by the International Bureau). \boxtimes has been communicated by the International Bureau. is not required, as the application was filed in the United States Receiving Office (RO/US). c. An English language translation of the International Application under PCT Article 19 (35 U.S.C. 371(c)(2)). is attached hereto. " has been previously submitted under 35 U.S C. 154(d)(4). b. Amendments to the claims of the International Application under PCT Article 19 (35 U.S C. 371(c)(3)). \times are attached hereto (required only if not communicated by the International Bureau) have been communicated by the International Bureau. b. have not been made; however, the time limit for making such amendments has NOT expired. c. $|\mathbf{X}|$ have not been made and will not be made. d. An English language translation of the amendments to the claims under PCT Article 19 (35 U S C. 371(c)(3)) П An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 10. Items 11. to 16. below concern document(s) or information included: An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 11. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 12. \boxtimes A FIRST preliminary amendment. 13 A SECOND or SUBSEQUENT preliminary amendment 14 A substitute specification. A change of power of attorney and/or address letter. A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U S.C. 1821 - 1.825. 17 A second copy of the published international application under 35 U S.C. 154(d)(4) 18 A second copy of the English language translation of the international application under 35 U.S C. 154(d)(4) 19 Other items or information: return receipt postcard. 20. \times CERTIFICATE OF MAILING BY "EXPRESS MAIL" Date of Deposit: September 11, 2001 Express Mail Label No.: EL 719486359 US I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, BOX PCT, Washington, D.C. 20231. a Durant

JC16 Rec'd PCT/PTO SEP 1 1 INTERNATIONAL ATTORNEY'SDOCKET U.S. APPLICATION NO. (if known, see 37 CFR 15) & 87 NUMBER: 280502000200 APPLICATION NO. PCT/CA00/00266 CALCULATIONS 21. The following fees are submitted: PTO USE ONLY BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....\$1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....\$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provision of PCT Article 33(1)-(4)\$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)\$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT = \$860.00 Surcharge of \$130.00 for furnishing the oath or declaration later than □ 20 ⋈ 30 months from \$130.00 the earliest claimed priority date (37 CFR 1.492(e)). RATE NUMBER FILED **NUMBER EXTRA CLAIMS** \$306.00 37 - 20 =17 x \$18.00 Total claims 0 3 - 3 = x \$80.00 \$ Independent claims + \$270.00 \$ MULTIPLE DEPENDENT CLAIM(S) (if applicable) TOTAL OF ABOVE CALCULATIONS = \$436.00 ☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. SUBTOTAL = Processing fee of \$130.00 for furnishing the English translation later than □ 20 □ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). \$860.00 TOTAL NATIONAL FEE = Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property \$1296.00 TOTAL FEES ENCLOSED = Amount to be refunded: charged: | \$1296.00 ☐ A check in the amount of \$___ to cover the above fees is enclosed. a. Please charge my **Deposit Account No. 03-1952** in the amount of \$1,296.00 to cover the above fees. A duplicate copy h. of this sheet is enclosed. The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to c. Deposit Account No. 03-1952. A duplicate copy of this sheet is enclosed. Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card d. information should not be included on this form. Provide credit card information and authorization on PTO-2038. NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO: Karen Babyak Dow Karen B. Dow Morrison & Foerster LLP

Karen B. Dow

Registration No. (29,684)

Suite 500

3811 Valley Centre Drive

San Diego, California 92130-2332

Docket No. 280502000200 International Application No. PCT/CA00/00266

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D.C. 20231.

Nora Durant

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Chaim M. ROIFMAN, et al.

Serial No.:

Not Yet Assigned

Filing Date:

Herewith

International Application No. PCT/CA00/00266

International Filing Date March 13, 2000

For:

METHODS AND COMPOSITIONS FOR

TREATING LEUKEMIA

Examiner: Not Yet Assigned

Group Art Unit: Not Yet Assigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents BOX PCT Washington, D.C. 20231

Dear Sir:

This is a preliminary amendment prior to examination, please amend the application as follows:

In the Specification:

On page 1 of the specification, under the title, please amend by inserting the following:

. PATENT Docket No. 280502000200 International Application No. PCT/CA00/00266

--This application claims priority to PCT application PCT/CA00/00266 filed March 13, 2000, which claims priority to Canadian Application No. 2,265,396, filed March 12, 1999.--

Please create a new page of the specification for the abstract as follows:

-- Compounds of general formula (I), wherein R_1 is H or C1 to C3 alkyl; R_2 is aryl or $-(CH_2)_n$ -aryl and n is 1 to 4; R_3 is H or CH_3 ; and R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole are described, as well as methods for treating cell proliferative disorders and neoplastic disorders.--

In the Claims:

Please replace Claims 34-36 as follows:

(Amended) 34. A method in accordance with claim 23 wherein the neoplastic disorder is a lymphoma, a leukemia or a metastatic carcinoma.

(Amended) 35. A method in accordance with claim 34 wherein the neoplastic disorder is Acute Lymphoblastic Leukemia.

(Amended) 36. A method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound in accordance with claim 1.

Enclosed is the following Exhibit A:

Exhibit A: Marked-up Version of Amendments to the Claims.

Remarks

The claims have been amended to eliminate multiple claim dependencies. The changes to the claims are editorial and do not constitute new matter. Entry of the amendment is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition

D9936897 M119602

PATENT Docket No. 280502000200 International Application No. PCT/CA00/00266

for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 280502000200. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: September // , 2001

By:

Registration No. 29,684

Morrison & Foerster LLP 3811 Valley Centre Drive

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San Diego, California 92130-2332

Telephone: (858) 720-7960 Facsimile: (858) 720-5125

PATENT
Docket No. 280502000200
International Application No. PCT/CA00/00266

EXHIBIT A

MARKED-UP VERSION OF AMENDMENTS TO THE CLAIMS

(Amended) 34. A method in accordance with [any one of claims 23 to 33] claim 23 wherein the neoplastic disorder is a lymphoma, a leukemia or a metastatic carcinoma.

(Amended) 35. A method in accordance with [any one of claims 23 to 33] claim 34 wherein the neoplastic disorder is Acute Lymphoblastic Leukemia.

(Amended) 36. A method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound in accordance with [any one of claims 1 to 11] claim 1.

Docket No. 280502000200

International Application No. PCT/CA00/00266

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Nora Durant

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Chaim M. ROIFMAN, et al.

Serial No.:

Not Yet Assigned

Filing Date:

Herewith

International Application No. PCT/CA00/00266

International Filing Date March 13, 2000

For:

METHODS AND COMPOSITIONS FOR

TREATING LEUKEMIA

Examiner: Not Yet Assigned

Group Art Unit: Not Yet Assigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents BOX PCT Washington, D.C. 20231

Dear Sir:

This is a preliminary amendment prior to examination, please amend the application as follows:

In the Specification:

On page 1 of the specification, under the title, please amend by inserting the following:

O9936557.alC602

. PATENT

Docket No. 280502000200 International Application No. PCT/CA00/00266

--This application claims priority to PCT application PCT/CA00/00266 filed March 13, 2000, which claims priority to Canadian Application No. 2,265,396, filed March 12, 1999.--

Please create a new page of the specification for the abstract as follows:

-- Compounds of general formula (I), wherein R₁ is H or C1 to C3 alkyl; R₂ is aryl or -(CH₂)_n-aryl and n is 1 to 4; R₃ is H or CH₃; and R₄ is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole are described, as well as methods for treating cell proliferative disorders and neoplastic disorders.--

In the Claims:

Please replace Claims 34-36 as follows:

(Amended) 34. A method in accordance with claim 23 wherein the neoplastic disorder is a lymphoma, a leukemia or a metastatic carcinoma.

(Amended) 35. A method in accordance with claim 34 wherein the neoplastic disorder is Acute Lymphoblastic Leukemia.

(Amended) 36. A method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound in accordance with claim 1.

Enclosed is the following Exhibit A:

Exhibit A: Marked-up Version of Amendments to the Claims.

Remarks

The claims have been amended to eliminate multiple claim dependencies. The changes to the claims are editorial and do not constitute new matter. Entry of the amendment is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition

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Docket No. 280502000200
International Application No. PCT/CA00/00266

for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. <u>280502000200</u>. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated:

September // , 2001

Bv:

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. PATENT Docket No. 280502000200 International Application No. PCT/CA00/00266

EXHIBIT A

MARKED-UP VERSION OF AMENDMENTS TO THE CLAIMS

(Amended) 34. A method in accordance with [any one of claims 23 to 33] claim 23 wherein the neoplastic disorder is a lymphoma, a leukemia or a metastatic carcinoma.

(Amended) 35. A method in accordance with [any one of claims 23 to 33] claim 34 wherein the neoplastic disorder is Acute Lymphoblastic Leukemia.

(Amended) 36. A method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound in accordance with [any one of claims 1 to 11] claim 1.

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METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA

Field of the Invention

This invention relates to tyrphostins or benzylidene malononitrile compounds which are useful as antiproliferative pharmaceuticals for treating a variety of cell proliferative disorders.

Background of the Invention

A number of tyrphostins or benzylidene malononitrile derivatives have been described which are tyrosine kinase inhibitors and are effective to inhibit cell proliferation, for example in human leukemia (United States Patents Nos. 5,217,999 and 5,773,476).

Summary of the Invention

The present invention provides a new group of tyrphostins or benzylidene malononitrile derivatives of improved effectiveness as inhibitors of cell growth.

In accordance with one embodiment, the compounds of the invention have the general formula:

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wherein

R, is H or C1 to C3 alkyl;

30 R_2 is aryl or $-(CH_2)_n$ - aryl and n is 1 to 4;

R₃ is H or CH₃; and

R₄ is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

In accordance with a further embodiment, the compounds have the general formula I above, wherein

R₁ is H, methyl or ethyl;

 R_2 is phenyl, benzyl, $-(CH_2)_2$ -phenyl, $-(CH_2)_3$ -phenyl or 2-thiobenzothiazole:

R₃ is H; and

10 R₄ is phenyl.

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In accordance with a preferred embodiment, the compounds are those shown in Figures 2 to 6.

In accordance with a further embodiment, the invention provides a pharmaceutical composition comprising as active ingredient a compound of formula I above.

In accordance with a further embodiment, the invention provides a pharmaceutical composition comprising as active ingredient one of the compounds shown in Figures 2 to 6.

In accordance with a further embodiment, the invention provides a method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound of formula I above.

In accordance with a further embodiment, the invention provides a method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of at least one of the compounds shown in Figures 2 to 6.

In accordance with a further embodiment, the invention provides a method for treating a neoplastic disorder in a mammal comprising administering to the mammal an effective amount of a compound of formula I above.

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In accordance with a preferred embodiment, the invention provides a method for treating acute lymphoblastic leukemia (ALL) in a mammal comprising administering to the mammal an effective amount of a compound of formula I above.

In accordance with a further preferred embodiment, the invention provides a method for treating acute lymphoblastic leukemia (ALL) in a mammal comprising administering to the mammal an effective amount of at least one of the compounds shown in Figures 2 to 6.

10 Summary of the Drawings

Figure 1 shows in schematic form a process for synthesising the compounds of the invention. R_1 is C1 to C3 alkyl; R_2 is aryl or $-(CH_2)_n$ -aryl, where n is 1 to 4; R_3 is H or CH_3 ; and R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

Figures 2 to 6 show some examples of the compounds of the invention.

Figure 7 shows the inhibitory effect (expressed as colony formation/1.5 \times 10⁵ cells) of several compounds of the invention on growth of G2 ALL cells.

Figure 8 shows the inhibitory effect of several compounds of the invention on growth of G2 ALL cells.

Figures 9, 10, 11 and 12 show the effect of compounds AG 1977, AG 1978, AG 2009 and AG 2010 respectively on the growth of normal BM cells, as indicated by three different assays.

Figure 13 shows inhibition of G2 ALL cells by various concentrations of the compounds of the invention.

Figure 14 shows a dose response curve of G2 ALL cell inhibition and AG 2009 concentration.

Figure 15 shows inhibition C1 ALL cells by various concentrations of AG 1977 and 1978.

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Figure 16 shows inhibition of A1 ALL cells by various concentrations of the compounds of the invention.

Figure 17 shows inhibition of blast cells by several compounds of the invention.

5 <u>Detailed Description of the Invention</u>

A number of tyrphostins, including the compound α -cyano-3,4-dihydroxy-cinnamal benzylamide (AG 490), have previously been shown to inhibit the growth of leukemia cells, and to be useful as active ingredients in pharmaceutical compositions for treating leukemia.

The present inventors have found that a new group of substituted benzylidene malononitriles have unexpectedly improved efficacy for treatment of cell proliferative diseases. For example, the inventors have shown that the tyrphostins described herein gave complete suppression of proliferation of human acute lymphoblastic leukemia (ALL) cells and of pre-B ALL blast cells without significantly affecting normal bone marrow cells, as described in the examples.

The compounds of the present invention have the formula:

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$$R_1O$$
 CN
 R_2
 R_3
 R_4

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wherein

R₁ is H or C1 to C3 alkyl;

 R_2 is aryl or $-(CH_2)_n$ - aryl and n is 1 to 4;

30 R₃ is H or CH₃; and

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R₄ is substituted or unsubstituted phenyl, pyridyl; thiophene, furan, indole, pyrrole, thiazole or imidazole.

The compounds of the invention are prepared by the process shown schematically in Figure 1. The required aldehydes (a) are available commercially or can be synthesised as previously described (Gazit et al., (1993), J. Med. Chem., v. 36, p. 3556). Benzyl cyano acetamide (b) is synthesised as described previously (Gazit et al., (1991), J. Med. Chem., v. 34, p. 1896).

A preferred group of compounds are the compounds shown in Figures 2 to 6.

The compounds of the invention may be used to treat a variety of neoplastic disorders, including leukemia, lymphomas, metastatic carcinomas and other forms of cancer. Leukemias which may be treated include B-lineage Acute Lymphoblastic Leukemia (ALL), such as the aggressive Philadelphia* leukemia, and acute myelocytic leukemia and juvenile myelo monocytic leukemia; lymphomas which may be treated include B-lineage Burkitt's lymphoma and Non-Hodgkin's lymphomas, such as the Ki-1 positive anaplastic large cell lymphomas.

The compounds of the invention may also be used to reduce or inhibit cell growth in a variety of cell proliferative disorders such as inflammatory disorders, allergic disorders, autoimmune diseases and graft rejection situations in which cell growth suppression, and preferably T cell growth suppression, is desired.

The compounds of the invention may also be used to inhibit the activity of Jak2 kinase. They may therefore be used to treat any disorder associated with increased or undesired Jak2 kinase activity.

The compounds of this invention may be used in the form of the free base, in the form of salts and as hydrates. All forms are within the scope of the invention. Acid addition salts may be formed and provide a more convenient form for use; in practice, use of the salt form inherently amounts to

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use of the base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the animal organism in pharmaceutical doses of the salts, so that the beneficial properties inherent in the free base are not vitiated by side effects ascribable to the anions. Although pharmaceutically acceptable salts of the basic compounds are preferred, all acid addition salts are useful as sources of the free base form even if the particular salt per se is desired only as an intermediate product as, for example, when the salt is formed only for the purposes of purification and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures.

Pharmaceutically acceptable salts within the scope of the invention include those derived from the following acids; mineral acids such as hydrochloric acid, sulfuric acid, phosphoric acid and sulfamic acid; and organic acids such as acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, quinic acid, and the like.

Compounds may be examined for their efficacy in inhibiting cell growth in cell proliferation assays such as those described herein.

In accordance with the methods of the invention, the described tyrphostins may be administered to a leukemia patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compositions of the invention may be administered orally or parenterally, the latter route including intravenous and subcutaneous administration. Parenteral administration may be by continuous infusion over a selected period of time.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may

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be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

The active compound may also be administered parenterally or intraperitoneally. Solutions of the active compound as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersion can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

The therapeutic compounds of this invention may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers, as noted above, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

Tyrphostins are administered initially in a suitable dosage to provide a blood level of about 10 μ M. The dosage may be adjusted as required, depending on the clinical response.

An alternate therapeutic approach is to obtain bone marrow or peripheral blood cells containing stem cells from patients with leukemia or lymphoma and to treat these cells <u>ex vivo</u> with a tyrphostin of the invention to purge or kill leukemia or lymphoma cells present with minimal inhibition of normal stem cells. The treated cells are washed to remove excess tyrphostin and returned to the patient.

For such <u>ex vivo</u> treatment of cells over a short period, for example around 5 hours, higher doses of tyrphostin may be used than for long term <u>in vivo</u> therapy; for example, concentrations of 50µM or higher may be used.

5 <u>Examples</u>

The Examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

10 Example 1 - Synthesis of Compounds

The compounds of the invention were synthesised generally by the process shown schematically in Figure 1.

Compound AG 1946: N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide

 $R_1 = CH_3$, $R_2 = benzyl$, $R_3 = H$, $R_4 = phenyl$

(a) 207 mg 0.72mM 4-hydroxy-3-methoxy-5-methylene thiobenzyl benzaldehyde, 130 mg 0.75 mM N-benzyl cyano acetamide and 10 mg β-alanine in 25 mL ethanol were refluxed 3 hours. Evaporation and trituration with dichloromethane-hexane gave 305 mg yellow-green solid, 95% yield, mp-135°.

NMR (acetone d_6) δ 8.17(1H,s,vinyl), 7.70(1H,d,J=2.1 Hz), 7.57(1H,d,J=2.1 Hz), 7.3(10H,m), 4.60(2H,s), 3.93(3H,s), 3.78(2H,s), 3.74(2H,s).

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Compound AG 1977: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide

 $R_1 = H$, $R_2 = benzyl$, $R_3 = H$, $R_4 = phenyl$

(b) To 450 mg of product from (a) in 30 mL dichloromethane was added 0.5 mL BBr₃. After stirring 1 hour at room temperature, water

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was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 340 mg, 78% yield. yellow solid, mp-195°.

NMR (acetone d₆) δ 8.08(1H,s,vinyl), 7.62(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 3.78(2H,s), 3.75(2H,s).

MS m/e-430(M⁺,16%), 175(100%).

Compound AG 1951: N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide

10 $R_1 = CH_2CH_3$, $R_2 = phenyl$, $R_3 = H$, $R_4 = phenyl$

- (c) 500 mg 1.74mM 3-ethoxy-4-hydroxy-5-methylene thiophenyl benzaldehyde, 310 mg 1.78 mM N-benzyl cyano acetamide and 25 mg β-alanine in 40 mL ethanol were refluxed 4 hours. Evaporation and trituration with hexane gave 730 mg yellow solid, 95% yield, mp-108°.
- 15 NMR (acetone d_6) δ 8.10(1H,s,vinyl), 7.70(1H,d,J=2.2 Hz), 7.53(1H,d,J=2.2 Hz), 7.3(10H,m), 4.58(2H,d,J=6.0 Hz), 4.26(2H,s), 4.18(2H,q,J=7.0 Hz), 1.42(3H,t,J=7.0 Hz).

Compound AG 1978: N-benzyl-2-cyano-3-(3',4-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide

 $R_1 = H$, $R_2 = phenyl$, $R_3 = H$, $R_4 = phenyl$

(d) To 200 mg of product from (c) in 30 mL dichloromethane was added 0.4 mL BBr₃. After stirring 1 hour at room temperature water was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 91 mg, 47% yield, yellow solid, mp-175°.

NMR (acetone d_6) δ 8.01(1H,s,vinyl), 7.63(1H,d,J=2.2 Hz), 7.3(11H.m), 4.58(2H,s), 4.26(2H,s).

MS m/e- 416(M*,16%), 309(12), 263(32), 196(37), 175(100%).

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Compound AG 2007: N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide

 $R_1 = CH_3$, $R_2 = CH_2CH_2Ph$, $R_3 = H$, $R_4 = phenyl$

(e) 400 mg 1.3 mM 4-hydroxy-3-methoxy-5-methylene thiophenethyl benzaldehyde, 240 mg 1.38 mM N-benzyl cyano acetamide and 20 mg β -alanine in 40 mL ethanol were refluxed 4 hours. Evaporation and trituration with dichloromethane- hexane gave 450 mg yellow solid, 88% yield, mp-102°.

NMR (CDC1₃) δ 8.25(1H,s,vinyl), 7.62(1H,d,J=2.2 Hz), 7.3(11H,m), 4.61(2H,d,J=6.0 Hz), 3.96(3H,s), 3.81(2H,s), 2.80(4H,m).

Compound AG 2009: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide

 $R_1 = H_1$, $R_2 = CH_2CH_2Ph_1$, $R_3 = H_1$, $R_4 = phenyl$

- 15 (f) To 320 mg of product from (e) in 25 mL dichloromethane was added 0.3 mL BBr₃. After stirring 1 hour at room temperature water was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 110 mg, 35% yield, yellow solid, mp-153°.
- 20 NMR (acetone d_6) δ 8.09(1H,s,vinyl), 7.63(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 3.85(2H,s), 2.80(4H,m).

Compound AG 2008: N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide

25 $R_1 = CH_3$, $R_2 = CH_2CH_2CH_2Ph$, $R_3 = H$, $R_4 = phenyl$

(g) 800 mg 2.5 mM 4-hydroxy-3-methoxy-5-methylene thiopropylphenyl benzaldehyde, 430 mg, 2.5 mM N-benzyl cyano acetamide and 20 mg β-alanine in 40 mL ethanol were refluxed 4 hours. Evaporation and trituration with Cc1₄ gave 760 mg yellow solid, 63% yield, mp-78°.

NMR (CDC1₃) δ 8.25(1H,s,vinyl), 7.62(1H,d,J=2.2 Hz), 7.3(11H,m), 4.61(2H,d,J=6.0 Hz), 3.96(3H,s), 3.81(2H,s), 2.69(2H,t,J=6.0 Hz). 2.50(2H,t,J=6.0 Hz), 1.90(2H,quint.,J=6.0 Hz).

Compound AG 2010: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide

 $R_1 = H$, $R_2 = CH_2CH_2CH_2Ph$, $R_3 = H$, $R_4 = phenyl$

(h) To 660 mg of product from (g) in 25 mL dichloromethane was added 0.6 mL BBr₃. After stirring 1 hour at room temperature water was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 610 mg, 95% yield. yellow solid, mp-138°.

NMR (acetone d_6) δ 8.17(1H,s,vinyl), 7.63(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 3.80(2H,s), 2.69(2H,t,J=6.0 Hz), 2.52(2H,t,J=6.0 Hz), 1.90(2H quint J=6.0 Hz)

1.90(2H,quint.,J=6.0 Hz).

Compound AG 1976: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide $R_1 = H$, $R_2 = 2$ -thiobenzothiazole, $R_3 = H$, $R_4 = phenyl$

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- (i) 330 mg, 1 mM, 4-hydroxy-3-methoxy-5-methylene (2-thiobenzothiazole) benzaldehyde, 180 mg, 1.03 mM, N-benzyl cyano acetamide and 15 mg β -alanine in 20 ml ethanol were refluxed 4 hours. Cooling and filtering gave 460 mg, 95% yield, yellow solid.
- 25 (j) To 200 mg, 0.4 mM, solid from (i) in 30 ml dichloromethane was added 0.4 ml BBr₃. After stirring 1 hour at room temperature, water and 3 ml HCl was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 40 mg, 20% yield, bright yellow solid, mp-225%.

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NMR Acetone $d_6 \delta 8.07(1H,s,vinyl)$, 7.95(2H,m), 7.6 7.1(9H,m), 4.60(2H,s), 4.48(2H,d,J=5.9 Hz).

Example 2 - Inhibition of Colony Formation - Acute Lymphoblastic Leukemia (ALL) Cell Lines

Inhibition of colony formation was studied by methods described previously (Kamel-Reid et al., (1992), Leukemia, v. 6, pp. 8-17; Meydan et al., (1996), Nature, v. 379, pp. 645-648).

ALL cell lines A1 (at 8x10⁵ cells/ml), C1 (at 4x10⁴ cells/ml) and G2 (at 1.15x10⁶ cells/ml) were plated in 1 ml volumes, in the absence of exogenous growth factors, into 35 mm petri dishes (Nunc, Gibco) containing alpha MEM (Gibco) plus 10% FCS (Cansera Rexdale, Ont.) in 0.9% (vol/vol) methylcellulose (Fluka, Switzerland). Cultures were set up at 37°C with 5% CO₂ in a humidified atmosphere and 10 uM of a selected tyrphostin was added. Colonies consisting of more than 20 cells were counted at 12 days (A1), 5 days (C1) and 14 days (G2) using an inverted microscope. The results with G2 are shown in Figure 7. Similar results were obtained with A1 and C1.

20 Example 3 - Effect on Bone Marrow Cells

Compounds showing inhibition of ALL colony formation were examined for their effect on normal bone marrow cells using a modified CFU-GEMM clonogenic assay.

The assay was performed according to Fauser and Messner (1978),

Blood, v. 52, pp. 1243-8, and Messner and Fausser (1980), Blut, v. 41, pp.

327-333, with some variations. In brief, heparinized bone marrow cells were layered over Percoll (Pharmacia Fine Chemical, Piscataway N.J.) at a density of 1.077 gm/ml and centrifuged at 400 g at 4°C for 10 min. to remove neutrophils and RBCs. The fractionated bone marrow cells at 2x10⁵ cells/ml were cultured in IMDM (OCI, Toronto) containing 0.9% (vol/vol)

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methylcellulose supplemented with 30% FCS (Cansera Rexdale, Ont.) or normal human plasma, a cocktail of cytokines consisting of G-CSF (10 ng/ml. Amgen), IL-3 (40 U/ml, Immunex), MGF (50 ng/ml, Immunex), Erythropoietin (2u/ml, Epprex) or TPO (10 ng/ml, Amgen) and $5x10^{-5}$ 2-mercaptoethanol. The culture mixture was plated in 1 ml volumes into 35 mm petri dishes and incubated at 37° C with 5% CO₂ in a humidified atmosphere with concentrations of tyrphostin up to $40~\mu m$. The results are shown in Figures 9 to 12.

The BFU-E's (erythroid colonies) and the CFU-GEMM (mixed colonies) exhibited inhibition at and above $25\mu M$ (Fig. not shown), while the CFU-C's (granulocytes, monocytes and macrophages) showed a dramatic increase of colony proliferation peaking at $25\mu M$ and a reduction by $50\mu M$ (Fig. not shown). AG 2010 showed significant inhibition at $40\mu M$, while the remaining compounds showed mild to significant inhibition of erythroid and mixed colonies followed by the myeloid population at $20\mu M$.

Example 4 - Inhibition of ALL Cells

Various concentrations of tyrphostins were tested for inhibition of ALL cells in the clonogenic assay described in Example 2. Compounds AG 1977. 1978, 2007, 2008, 2009 and 2010 were tested against ALL cell lines A1. C1 and G2 in doses ranging from nanomolar to micromolar values. The results are shown in Figures 8, 13, 15 and 16.

AG 2009 demonstrated the most potent clonogenic inhibition, in a dose responsive manner, against G2 cells (Fig. 13). It showed a greater than 50% inhibition at a dose of 16nM and a differential therapeutic index of greater than 2 logs in a survival curve (Fig. 14) of normal BM and G2 colonies.

Example 5 - Inhibition of Blast Cells

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The compounds were further tested in an ALL blast colony assay, against bone marrow samples from two patients with pre –B ALL phenotype based on their FAB classification.

The ALL blast colony assay was performed as described previously (Estrov, Z. et al., (1988), Cancer Res., v. 48, p. 5901) with some modifications. Briefly, heparinized bone marrow cells were layered over Percoll (density 1.077 q/l; Pharmacia Fine Chemicals Piscataway, N.J.) and centrifuged (400g) for 10 minutes at 4°C to remove neutrophils and RBC's. The collected interphase fraction was further enriched for lymphoblasts before plating by using a magnetic cell separator, mini MACS, with separation column (Miltenyi Biotec Inc., 1250 Oakmead Park, Sunnyvale, Ca). With this procedure, ALL blasts were specifically isolated from the marrow mononuclear cell fraction using directly labelled MACS CD19 Microbeads monoclonal anti-human CD19 (Mouse IgG1, Kappa- Miltenyi Biotec Inc.) and/or indirect magnetic cell labeling using primary biotinylated antibody-(mouse anti-human CD10 monoclonal antibody, Caltag Laboratires, Ca.) and Streptavidin Microbeads (Miltenyi Biotech Inc.). The resulting cell population was composed of 99% lymphoblasts. The positively sorted cells were then cultured at 2x10⁵ cells/ml in alpha MEM (GIBCO) containing 0.9% (vol/vol) methylcellulose supplemented with 10% FCS (Cansera, Rexdale, Ont.). Irradiated autologous leukemic blasts were used as feeder cells (at 3x105 cells/ml). Cytokines, normally used, were deleted so that only spontaneous proliferation was evident.

The culture mixture was plated into 35mm petri dishes (Nunc, GIBCO) containing 1ml volumes and incubated at 37°C with 5% CO₂ in a humidified atmosphere. Colonies containing more than 20 cells were scored, using inverted microscope, at 5-7 days.

In both cases, significant inhibition of blast colonies was observed (See for example, Figure 17).

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Example 6 - Inhibition of Jak2 Kinase Activity

Compounds chosen on the basis of their ability to inhibit the growth (colony formation) of the pre-B leukemia cell line G2 were tested for inhibition of Jak2 kinase.

Jak2 kinase was immunoprecipitated from a 1% Triton-X100 lysate of 10x10⁶ G2 cells. An *in vitro* kinase assay was performed on the immunoprecipitated Jak2 in the presence or absence of varying concentrations of the compounds AG 1977, AG 1978, AG 2009 and AG 2010.

Stock solutions of 100mM tyrphostin were made in 100% DMSO and further dilutions made in 10% DMSO. Control kinase assays carried out in the presence of DMSO concentrations of 5-30% alone were unaffected by its presence.

Initial experiments were done with tyrphostin concentrations of 0.1, 1.0 and $10\mu M$. These concentrations had no affect on the kinase activity of Jak2. Using higher concentrations, 5, 25 and $50\mu M$, inhibition could be seen, as shown in Table 1 below. These results were obtained by scanning autoradiographs of the kinase assays.

TABLE 1
%inhibition of kinase activity

		Concentration		
	Tyrphostin	5μΜ	25μΜ	50µM
25				
	AG 1977	23	14	15
	AG 1978	10	13	46
	AG 2009	0	0	8
	AG 2010	5	5	39

The present invention is not limited to the features of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

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WE CLAIM:

1. A compound of the general formula:

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wherein

R₁ is H or C1 to C3 alkyl;

 R_2 is aryl or $-(CH_2)_n$ - aryl and n is 1 to 4;

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R₃ is H or CH₃; and

R₄ is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

2. A compound in accordance with claim 1 wherein

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R₁ is H, methyl or ethyl;

 R_2 is phenyl, benzyl, $-(CH_2)_2$ -phenyl, $-(CH_2)_3$ -phenyl or 2-

thiobenzothiazole;

R₃ is H; and

R₄ is phenyl.

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3. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide.

- 4. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide.
- 5 5. A compound in accordance with claim1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide.
- 6. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide.
 - 7. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide.
- 15 8. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.
- A compound in accordance with claim 1 wherein the compound is N benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl)
 phenyl) acrylamide.
 - 10. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.
 - 11. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

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12. A pharmaceutical composition comprising a compound of the formula:

10 wherein

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R₁ is H or C1 to C3 alkyl;

 R_2 is aryl or $-(CH_2)_n$ - aryl and n is 1 to 4;

R₃ is H or CH₃; and

R₄ is substituted or unsubstituted phenyl, pyridyl, thiophene, furan,

indole, pyrrole, thiazole or imidazole

and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition in accordance with claim 12 comprising a compound of formula I, wherein

20 R, is H, methyl or ethyl;

 R_2 is phenyl, benzyl, $-(CH_2)_2$ -phenyl, $-(CH_2)_3$ -phenyl or 2-thiobenzothiazole;

R₃ is H; and

R₄ is phenyl.

25

14. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide.

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- 15. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide.
- 5 16. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide
- 17. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide.
 - 18. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide.
 - 19. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.
 - 20. A pharmaceutical composition in accordance with claim 12 wherein the compound is.N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.
- 21. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

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- 22. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) acrylamide.
- 5 23. A method for treating a neoplastic disorder in a mammal comprising administering to the mammal an effective amount of a compound of the formula:

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wherein

R, is H or C1 to C3 alkyl;

 R_2 is anyl or $-(CH_2)_n$ - anyl and n is 1 to 4;

 R_3 is H or CH_3 ; and

 R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

24. A method in accordance with claim 23 comprising administering aneffective amount of a compound of formula I wherein

R, is H, methyl or ethyl;

 R_2 is phenyl, benzyl, -(CH₂)₂-phenyl, -(CH₂)₃-phenyl or 2-thiobenzothiazole;

R₃ is H; and

30 R₄ is phenyl.

25. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide.

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- 26. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide.
- 27. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide.
- 28. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide.
 - 29. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide.
- 20 30. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.
- 31. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.
 - 32. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

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- 33. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

- 34. A method in accordance with any one of claims 23 to 33 wherein the neoplastic disorder is a lymphoma, a leukemia or a metastatic carcinoma.
- 35. A method in accordance with any one of claims 23 to 33 wherein the neoplastic disorder is Acute Lymphoblastic Leukemia.
 - 36. A method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound in accordance with any one of claims 1 to 11.

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37. A method in accordance with claim 36 wherein the cell proliferative disorder is selected from the group consisting of an inflammatory disorder, an allergic disorder, an autoimmune disease or graft rejection.

PCT





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With international search report.

(54) Title: METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA

(57) Abstract

Compounds of general formula (I), wherein R₁ is H or C1 to C3 alkyl; R2 is aryl or -(CH2)n-aryl and n is 1 to 4; R3 is H or CH3; and R4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole are described, as well as methods for treating cell proliferative disorders and neoplastic disorders.

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$$R_1O$$
 CHO
 R_1O
 R_2
 R_2
 R_3
 R_4
 R_4

HO
$$R_3$$
HO R_4
HO R_4
HO R_4
HO R_4

Figure 1

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Figure 2

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Figure 3

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AG 1978

Figure 4

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$$\begin{array}{c|c} H_3CO \\ \hline \\ HO \\ \hline \\ S \\ \hline \\ CN \\ H \\ \hline \\ H \\ \hline \\ O \\ \\ \end{array}$$

AG 2008

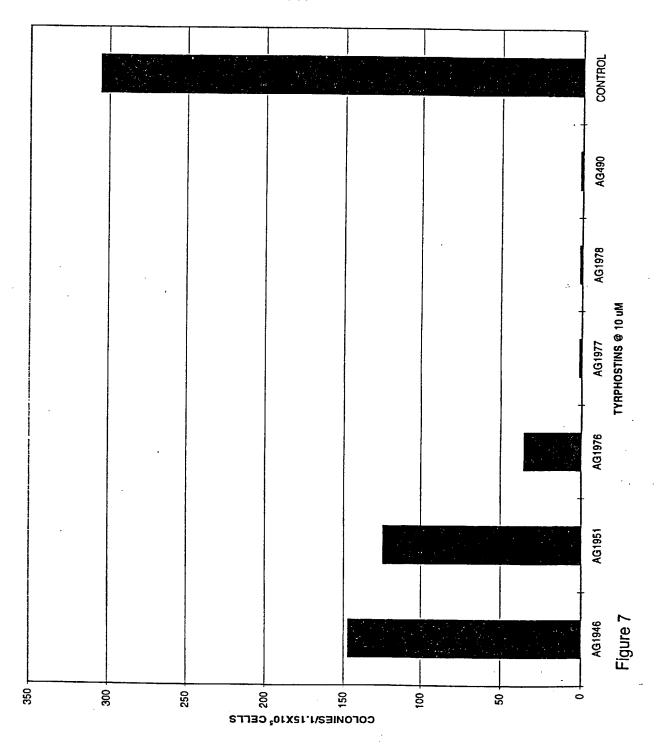
Figure 5

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Figure 6





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□ BFU-E

40

□ CFU-GEMM ■ CFU-C

200

0

0

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AG1977 µM

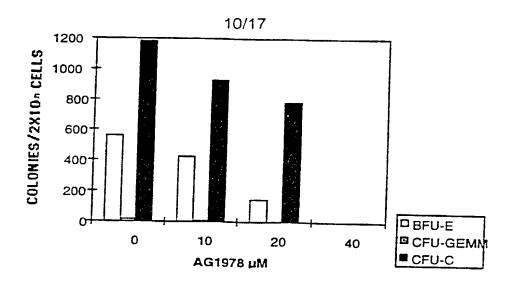


Figure 10

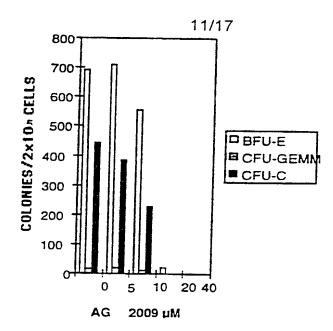


Figure 11

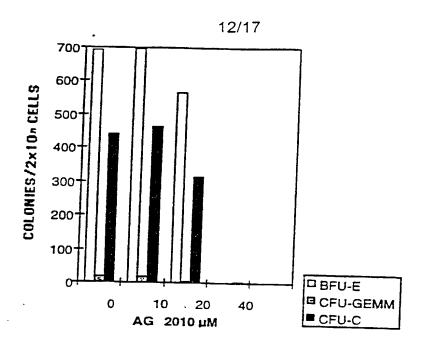
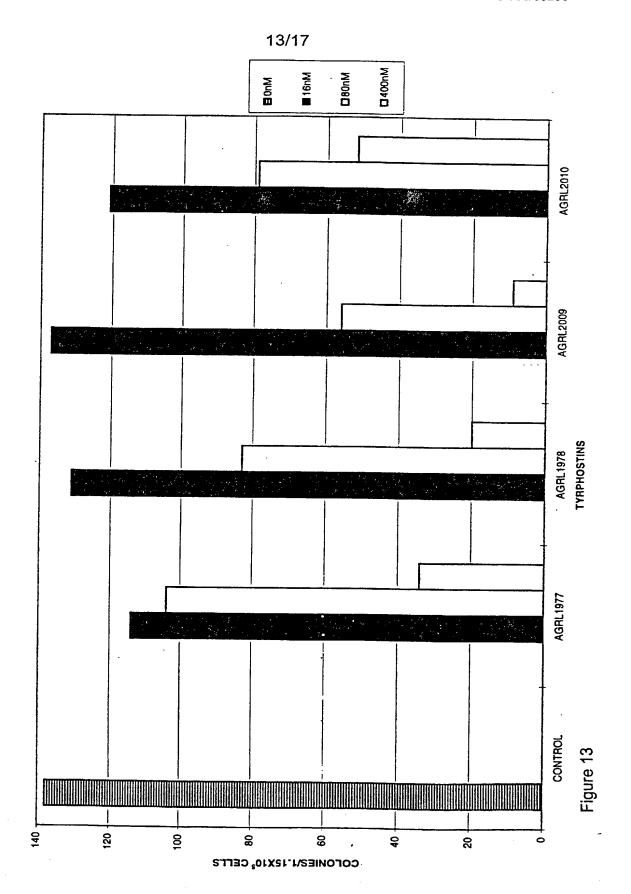


Figure 12



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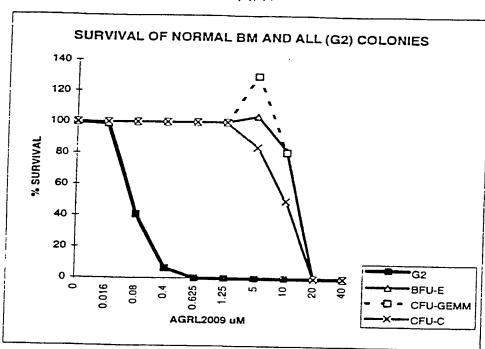
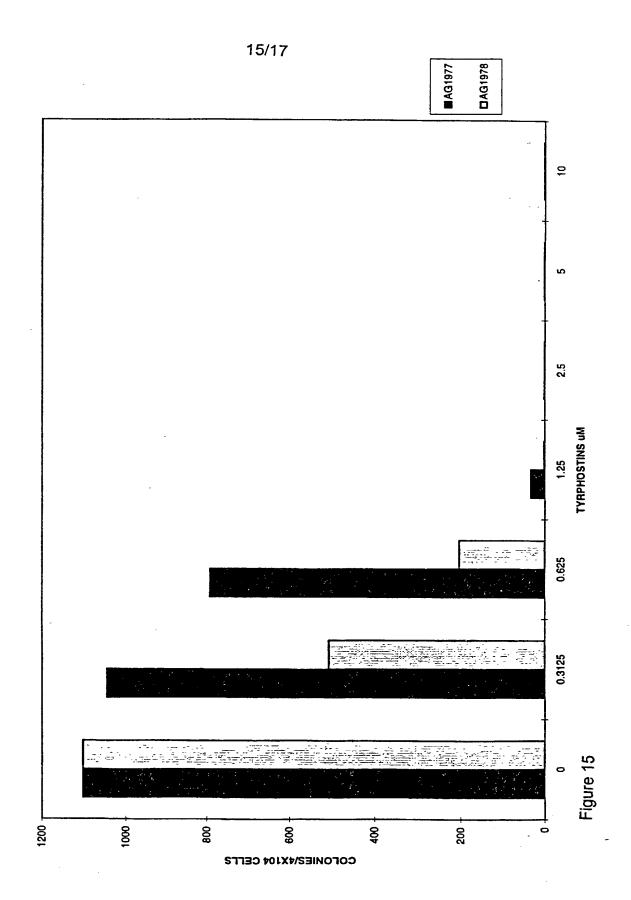
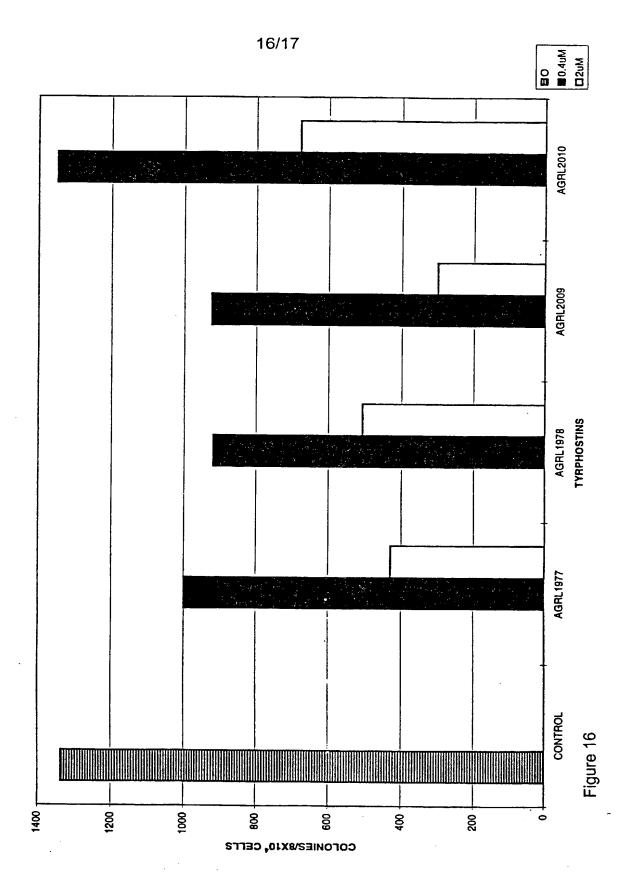


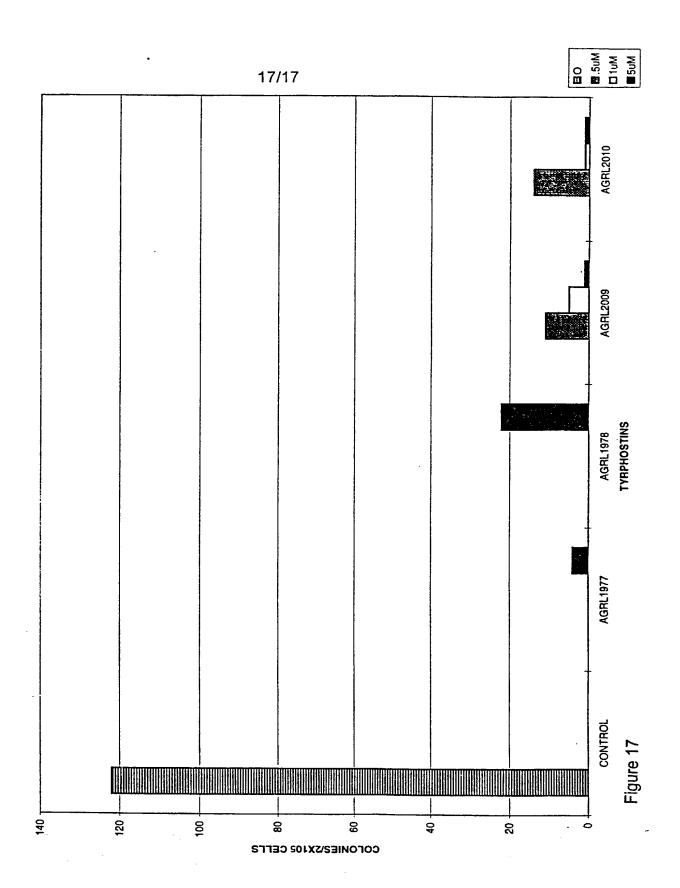
Figure 14



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PATENT Docket No. 28050200020

DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA, the specification of which is attached hereto unless the following box is checked:

was filed on September 11, 2001 as United States Application Serial No. 09/936,887 and on March 13, 2000 as PCT International Application No. PCT/CA00/00266.

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed	?
PCT/CA00/00266	PCT	13 March 2000	⊠Yes □No)
2,265,396	CA	12 March 1999		

I hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
*	

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I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to

patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status		
*		□Patented	□Pending	□Abandoned

I hereby appoint the following attorneys and agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

75-

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patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status		· 秦 秦 章 · 亦
*		□Patented	□Pending	□Abandoned

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PATENT Docket No. 28050200020

DECLARATION FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA, the specification of which is attached hereto unless the following box is checked:

was filed on September 11, 2001 as United States Application Serial No. 09/936,887 and on March 13, 2000 as PCT International Application No. PCT/CA00/00266.

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority (laimed?
PCT/CA00/00266	PCT	13 March 2000	⊠Yes	□No
2,265,396	CA	12 March 1999		

I hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
*	

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to

Frank Wu (Reg No. 41,386)
Peter J. Yim (Reg No. 44,417)
Karen R. Zachow (Reg No. 46,332)

David T. Yang (Reg No. 44,415) George C. Yu (Reg No. 44,418)

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Please direct all telephone calls to Karen B. Dow at (858) 720-7960.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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